Supplemental information

Quantifying differences

in iron deficiency-attributable anemia

during pregnancy and postpartum

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Additional File 1
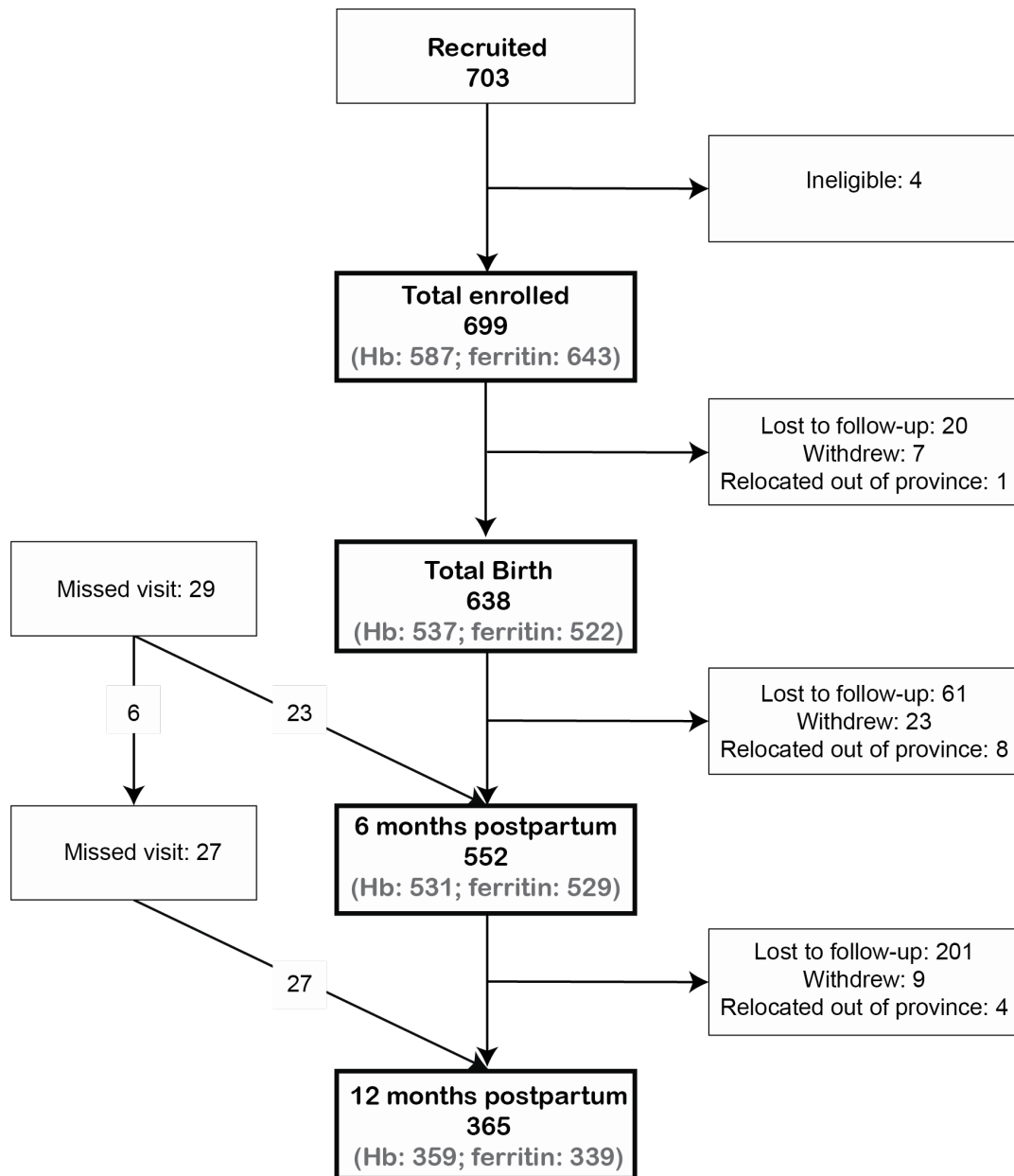
Supplementary Figure 1: Flow diagram of women enrolled and the subset followed to birth, 6 months postpartum and 12 months postpartum; and the haemoglobin (Hb) and ferritin measurements available at each visit. Related to Subject Details in STAR Methods.
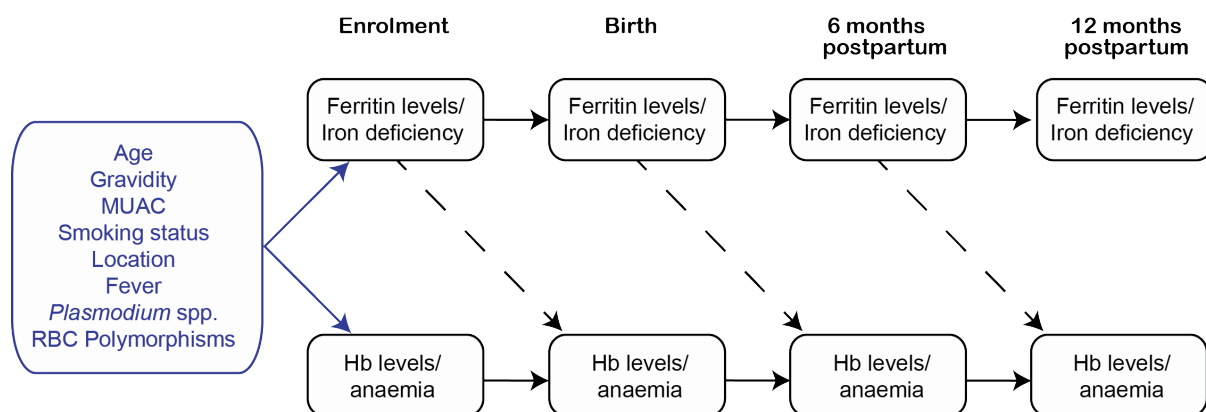
Supplementary Figure 2: Causal diagram depicts the relationships between ferritin levels/ iron deficiency and haemoglobin (Hb) levels/ anaemia at enrolment, birth, 6 months postpartum and 12 months postpartum. Related to Quantification and Statistical Analysis in STAR Methods. Potential confounders, presented as a single node in a blue box, include age, gravidity, mid-upper arm circumference (MUAC), location of enrolment clinic, smoking status, history of fever, *Plasmodium* spp. infection and red blood cell (RBC) polymorphisms. Angled dashed arrows depict associations between the preceding iron stores and current haemoglobin levels/anaemia (lagged effect) – the key associations of interest. Adherence to iron supplements was not included as it is not a common cause confounder and was only recorded at a single time-point (birth).

Supplementary Table 1: Enrolment cohort characteristics for the subset of women who returned at birth, and at 6 and 12 months postpartum. Related to Table 1.

Variable		Birth	6 months postpartum	12 months postpartum	
		Included (n=638)	Included (n=552)	Included (n=365)	Lost to follow-up (n=334)
Sociodemographics					
Enrolment Clinic	Vunapope	172 (27·0%)	155 (28·1%)	106 (29·0%)	78 (23·4%)
	Nonga	71 (11·1%)	63 (11·4%)	35 (9·6%)	48 (14·4%)
	Keravat	107 (16·8%)	92 (16·7%)	58 (15·9%)	67 (20·1%)
	Napapar	148 (23·2%)	123 (22·3%)	94 (25·8%)	64 (19·2%)
	Paparatava	140 (21·9%)	119 (21·6%)	72 (19·7%)	77 (23·1%)
Age (years)		26.0 (23·0-30·0); 17-49	26.0 (23·0-30·0); 17-49	26·0 (22·0-30·0);17-42	26·0 (23·0-30·0); 16-49
Gravidity	Primigravidae	154 (24·1%)	129 (23·4%)	98 (26·8%)	79 (23·7%)
	Multigravidae	484 (75·9%)	423 (76·6%)	267 (73·2%)	255 (76·3%)
Highest level education	Primary or less	292 (45·8%)	254 (46·1%)	167 (45·8%)	158 (47·4%)
Employment status	Not employed	483 (75·7%)	414 (75·0%)	271 (74·2%)	260 (77·8%)
Smoking status	Never smoked	386 (60·6%)	340 (61·6%)	228 (62·5%)	199 (59·9%)
	Current/past smoker	251 (39·4%)	212 (38·4%)	137 (37·5%)	133 (40·1%)
Bed net use	Owns bed net	479 (75·1%)	419 (75·9%)	266 (72·9%)	261 (78·4%)
	Net used last night	398 (62·4%)	351 (63·6%)	232 (63·6%)	208 (78·7%)
Clinical Measures					
Gestational age (weeks) [a]		29·8 (28·0-32·5); 26-40	29·8 (28·0-32·5); 26-40	29·8 (28·0-31·6); 26-40	29·8 (28·0-33·4); 26-39
MUAC (cm)		26·0 [3·0]; 13·5-43·7	26·0 [3·0]; 13·5-43·7	26·1 [2·8]; 14·5-37·5	25·8 [3·1]; 13·5-43·7
BMI (kg/m²)		25·3 [3·2]; 17·9-34·3	25·3 [3·2]; 17·9-34·3	25·2 [3·1]; 18·2-34·3	25·4 [3·2]; 17·9-34·3
Fever [b]		81 (12·9%)	70 (12·9%)	37 (10·2%)	54 (16·6%)

Data are mean [SD], range; median (25th-75thpercentile), range; or n (%).

[a] Gestational age was estimated from ANC fundal height measurements in women with fundal height measurements ≥24cm.

[b] Self-reported history of fever during the pregnancy prior to their first ANC appointment.

BMI: Body mass index. MUAC: Mid-upper arm circumference

Supplementary Table 1 continued: Enrolment cohort characteristics for the subset of women who returned at birth, and at 6 and 12 months postpartum Related to Table 1.

Variable		Birth Included (n=638)	6 months postpartum Included (n=552)	12 months postpartum Included (n=365)	12 months postpartum Lost to follow-up (n=334)
Diagnostic Measurements					
Haemoglobin level (g/l)		96·3 [14.7], 41-145	95·9 [14·3], 41-137	96·3 [13·9], 41-143	96·4 [15·1], 44-145
Anaemia [c]		443 (82·2%)	383 (82·9%)	245 (84·5%)	238 (80·1%)
Ferritin level (µg/l)		9·1 (5·3-17·7), 0·6-292·4	8·9 (5·3-16·9), 0·6-292·4	9·2 (5·1-18·2), 0·6-292·4	9·3 (5·4-19·2), 0·6-264·5
Iron status [d]	Iron deficient	417 (82·6%)	363 (83·1%)	236 (81·1%)	212 (81·5%)
	Iron replete	88 (17·4%)	74 (16·9%)	55 (18·9%)	48 (18·5%)
Plasmodium spp. (PCR)	Negative	485 (87·9%)	415 (87·7%)	266 (87·8%)	262 (87·9%)
	Positive	67 (12·1%)	58 (12·3%)	37 (12·2%)	36 (12·1%)
	P. falciparum	28 (5·1%)	29 (6·1%)	14 (4·6%)	17 (5·7%)
	P. vivax	32 (5·8%)	36 (7·4%)	18 (5·9%)	17 (5·7%)
	Mixed	7 (1·3%)	7 (1·5%)	5 (1·7%)	2 (0·7%)
Genetic polymorphisms					
α^+-thalassemia	Wildtype	512 (83·0%)	446 (84·0%)	292 (83·2%)	272 (84·5%)
	Heterozygous	84 (13·6%)	67 (12·6%)	49 (14·0%)	38 (11·8%)
	Homozygous	21 (3·4%)	18 (3·4%)	10 (2·8%)	12 (3·7%)
CR1 deficiency [e]	H/H	40 (6·4%)	34 (6·3%)	21 (5·8%)	22 (6·9%)
	H/L	232 (37·1%)	208 (38·4%)	133 (37·0%)	119 (37·1%)
	L/L	354 (56·5%)	299 (55·3%)	205 (57·1%)	180 (56·1%)
SAO	Normal	593 (94·6%)	513 (94·8%)	335 (93·1%)	310 (96·6%)
	SAO	34 (5·4%)	28 (5·2%)	25 (6·9%)	11 (3·4%)

Data are mean [SD], range; median (25th-75thpercentile), range; or n (%)

[c] Anaemia defined as haemoglobin <110g/l at birth and haemoglobin <120g/l at 6 and 12 months postpartum

[d] Women classified as iron deficient: ferritin <15µg/l in pregnancy and postpartum. Women classified as iron replete: ferritin ≥15µg/l and CRP≤10mg/l in pregnancy and ferritin ≥15µg/l and CRP≤5mg/l postpartum.

[e] Allele abbreviations correspond to CR1 red blood cell surface expression levels: H allele: high expression; L allele: low.

spp: species. RDT: Rapid Diagnostic Test. PCR: Polymerase Chain Reaction. CR1: Complement Receptor 1. SAO: Southeast Asian Ovalocytosis

Supplementary Table 2: Unadjusted and adjusted estimated mean differences in haemoglobin levels (g/l) over pregnancy and postpartum periods. Related to Figure 2.

Model Parameter	Unadjusted estimate (95% CI); p-value	Adjusted estimate [a] (95% CI); p-value
Estimated mean at enrolment	96·42 (95·15, 97·69)	98.94 (97.43, 100.45)
Estimated mean difference [b]		
Enrolment	Reference	Reference
Birth	2·59 (1·04, 4·14); 0·001	-0·44 (-2·37, 1·49); 0·65
6 months postpartum	15·29 (13·73, 16·85); <0·001	11·63 (9·62, 13·65); <0·001
12 months postpartum	12·17 (10·38, 13·95); <0·001	9·63 (7·38, 11·87); <0·001
Between-women SD	9·32	7·32
Within-woman SD	12·93	13·02

Unadjusted or adjusted estimated mean differences (95% CI), p-value, were derived from linear mixed-effects models with a random effect for the individual-specific intercept. CI- Confidence Interval.

[a] Adjusted for age, mid-upper arm circumference, gravidity, smoking status, location of enrolment clinic, history of fever, *Plasmodium* spp. infection and red blood cell polymorphisms (α^+-thalassemia, complement receptor 1 deficiency, Southeast Asian ovalocytosis).

[b] The estimated mean haemoglobin level at enrolment is derived with confounders set to mean values of continuous variables or the prevalence of categorical variables.

Model Parameter	Unadjusted estimate (95% CI); p-value	Adjusted estimate [a] (95% CI); p-value
Supplementary Table 3: Unadjusted and adjusted relative difference in the geometric mean of ferritin levels (µg/l) over pregnancy and postpartum periods. Related to Figure 2.		
Estimated geometric mean at enrolment (µg/l)	10·28 (9·62, 10·98)	5·87 (3·26, 10·61)
Ratio of geometric means [b]		
Enrolment	Reference	Reference
Birth	2·31 (2·13, 2·49); <0·001	2·30 (2·11, 2·50); <0·001
6 months postpartum	2·81 (2·60, 3·03); <0·001	2·81 (2·58, 3·05); <0·001
12 months postpartum	2·64 (2·41, 2·88); <0·001	2·60 (2·35, 2·87); <0·001
Between-women SD ($\log_2$ (µg/l))	0·57	0·54
Within-woman SD ($\log_2$ (µg/l))	0·65	0·65

Unadjusted or adjusted estimated ratio of geometric means (95% CI), p-value, were derived from linear mixed-effects regression models with a random effect for the individual-specific intercept. CI- Confidence Interval.

[a] Adjusted for age, mid-upper arm circumference, gravidity, smoking status, location of enrolment clinic, history of fever, *Plasmodium* spp. infection and red blood cell polymorphisms (α^+-thalassemia, complement receptor 1 deficiency, Southeast Asian ovalocytosis).

[b] The estimated geometric mean ferritin level at enrolment is derived with confounders set to mean values of continuous variables or the prevalence of categorical variables.

Supplementary Table 4: Associations between iron stores and enrolment confounders; and the outcome, moderate-to-severe anaemia, over the entire study period. Related to Table 2.

Variable		Moderate-to-severe anaemia [a]	
		Unadjusted odds ratio (95% CI); p-value	Adjusted odds ratio (95% CI); p-value
Iron stores			
Ferritin (log$_2$(µg/l)) [b]		0·65 (0·59, 0·72); <0·001	-
Iron deficiency [c]	Replete	Reference	Reference
	Deficient	3·61 (2·56, 5·09); <0·001	3·86 (2·64, 5·65); <0·001
At enrolment			
Age (years)		1·00 (0·98, 1·03); 0·99	0·99 (0·96, 1·02); 0·55
Gravidity	Primigravida	Reference	Reference
	Multigravida	1·66 (1·20, 2·29); 0·002	1·93 (1·23, 3·01); 0·004
Smoking status	Never	Reference	Reference
	Current/ past	1·18 (0·89, 1·56); 0·26	1·22 (0·87, 1·72); 0·26
MUAC (cm)		0·92 (0·87, 0·96); <0·001	0·93 (0·87, 1·00); 0·04
	>23cm	Reference	-
	≤23cm	1·46 (0·97, 2·19); 0·07	-
Fever [d]	No	Reference	Reference
	Yes	1·20 (0·80, 1·82); 0·38	1·31 (0·81, 2·13); 0·27
Plasmodium spp. (PCR)	Negative	Reference	Reference
	Positive	2·75 (1·73, 4·37); <0·001	2·45 (1·40, 4·29); 0·002
α$^+$- thalassemia	Wildtype	Reference	Reference
	Het/Hom	2·69 (1·83, 3·94); <0·001	3·03 (1·87, 4·91); <0·001
CR1 deficiency	H/H	Reference	Reference
	H/L	0·88 (0·48, 1·61); 0·68	0·99 (0·50, 1·97); 0·99
	L/L	0·89 (0·49, 1·59); 0·68	1·00 (0·51, 1·95); 0·99
SAO	Normal	Reference	Reference
	SAO	1·35 (0·70, 2·57); 0·37	1·19 (0·59, 2·39); 0·63

Odds ratios were derived from logistic mixed-effects models with a random effect for the individual-specific intercept. Adjusted models included enrolment variables listed in the table and time (enrolment, birth, 6 months and 12 months postpartum). CI- Confidence Interval.

[a] Moderate-to-severe anaemia defined as haemoglobin <100g/l in pregnancy and haemoglobin <110g/l in the postpartum period.

[b] Ferritin transformed to log base-2 due to positively skewed distribution, thus the odds ratio represents the change associated with a two-fold increase in ferritin.

[c] Iron deficient: ferritin <15µg/l in pregnancy and postpartum; iron replete: ferritin ≥15µg/l & CRP≤10mg/l in pregnancy and ferritin ≥15µg/l & CRP≤5mg/l postpartum.

[d] Self-reported history of fever during the pregnancy prior to their first antenatal care appointment.

Het: heterozygous. Hom: homozygous. CR1: complement receptor 1; H/H: high CR1 expression; H/L: intermediate CR1 expression; L/L: low CR1 expression. SAO: Southeast Asian ovalocytosis.

Supplementary Table 5: Associations between enrolment exposures of interest, ferritin levels and iron deficiency over the entire study period. Related to Table 2.

		Ferritin levels (µg/l)		Iron deficiency *	
		Unadjusted geometric mean ratio (95% CI); p-value	Adjusted geometric mean ratio (95% CI); p-value	Unadjusted odds ratio (95% CI); p-value	Adjusted odds ratio (95% CI); p-value
Gravidity	Primigravida	Reference	Reference	Reference	Reference
	Multigravida	-0·27 (-0·44, -0·10); 0·002	-0·43 (-0·64, -0·22); <0·001	1·74 (1·04, 2·90); 0·04	2·66 (1·41, 5·02); 0·002
Plasmodium spp. (PCR)	Negative	Reference	Reference	Reference	Reference
	Positive	0·31 (0·06, 0·55); 0·01	0·40 (0·13, 0·63); 0·003	0·50 (0·24, 1·04); 0·07	0·47 (0·22, 1·00); 0·05
α^+- thalassemia	Wildtype	Reference	Reference	Reference	Reference
	Het/Hom	-0·02 (-0·22, 0·18); 0·84	-0·08 (-0·29, 0·14); 0·49	1·30 (0·69, 2·43); 0·42	1·35 (0·71, 2·60); 0·36

Estimated mean ferritin differences and odds ratios were derived from linear and logistic mixed-effects models with a random effect for the individual-specific intercept. Adjusted models included gravidity, *Plasmodium* spp. infection, maternal age, smoking status, mid-upper arm circumference, birth clinic location, fever, genetic polymorphisms (α^+- thalassemia*, complement receptor 1 deficiency and Southeast Asian ovalocytosis), and time (enrolment, birth, 6 months and 12 months postpartum).

* Iron deficiency in pregnancy defined as ferritin <15µg/l; iron replete was defined as ferritin ≥15µg/l and CRP ≤10mg/l. Iron deficiency postpartum defined as ferritin <15µg/l; iron replete was defined as ferritin ≥15µg/l and CRP ≤5mg/l.

Het: heterozygous. Hom: homozygous.

Supplementary Table 6: Effect modification of the associations between iron measurements and haemoglobin levels (g/l) over the entire study period. Related to Table 2.

Potential effect modifier	Enrolment iron store	Adjusted estimated mean difference (95% CI) [†]	Likelihood ratio test p-value
Plasmodium spp. infection∫			
Negative	Iron deficient [*] vs. replete	-7·84 (-9·99, -5·70)	0·57
Positive		-9·39 (-14·54, -3·25)	
Gravidity			
Primigravida	Iron deficient [*] vs. replete	-6·16 (-9·87, -2·46)	0·23
Multigravida		-8·62 (-10·87, -6·36)	
α⁺-thalassemia genotype			
Wildtype	Iron deficient [*] vs. replete	-8·37 (-10·57, -6·18)	0·45
Heterozygous/Homozygous		-6·55 (-10·97, -2·12)	

Estimated mean differences were derived from multivariable linear mixed-effects models with random effects for the individual-specific intercept and interaction terms between the iron store and potential effect modifier. Likelihood ratio test p-values were derived from comparing the likelihood of the models with and without the interaction terms.

[†] Adjusted for age, mid-upper arm circumference, gravidity, smoking status, residence, history of fever, *Plasmodium* spp. infection and RBC polymorphisms ($α^+$-thalassemia, complement receptor 1 deficiency, Southeast Asian ovalocytosis).

∫ *Plasmodium* spp. infection detected by PCR at enrolment.

[*] Women classified as iron deficient: ferritin <15μg/l; replete: ferritin ≥15μg/l and CRP≤10mg/l in pregnancy

/ CRP≤5mg/l postpartum.

Supplementary Table 7: Effect modification of the associations between iron measurements and anaemia over the entire study period. Related to Table 2.

Potential effect modifier	Enrolment iron store	Adjusted odds ratio [†] (95% CI)		Likelihood ratio test p-value
Plasmodium spp. infection∫				
Negative	Iron deficient [*] vs. replete	4·46 (2·77, 7·43)		0·76
Positive		5·63 (1·29, 24·55)		
Gravidity				
Primigravida	Iron deficient [*] vs. replete	2·67 (1·14, 6·24)		0·14
Multigravida		5·30 (3·08, 9·12)		
α+- thalassemia genotype				
Wildtype	Iron deficient [*] vs. replete	5·17 (3·03, 8·81)		0·15
Heterozygous/Homozygous		2·06 (0·63, 6·74)		

Odds ratios were derived from logistic mixed-effects models with random effects for the individual-specific intercept and interaction terms between the iron store and potential effect modifier. Likelihood ratio test p-values were derived from comparing the likelihood of the models with and without the interaction parameters.

Anaemia defined as haemoglobin <110g/l in pregnancy and <120g/l postpartum.

[†] Adjusted for age, mid-upper arm circumference, gravidity, smoking status, residence, history of fever, *Plasmodium* spp. infection and RBC polymorphisms (α+-thalassemia, complement receptor 1 deficiency, Southeast Asian ovalocytosis).

∫ *Plasmodium* spp. infection detected by PCR at enrolment.

[*] Women classified as iron deficient: ferritin <15μg/l; replete: ferritin ≥15μg/l and CRP≤10mg/l in pregnancy

/ CRP≤5mg/l postpartum.

Supplementary Table 8: Time-varying contributions of iron deficiency to moderate-to-severe anaemia at enrolment, birth, 6 months postpartum and 12 months postpartum. Related to Table 3.

	Evaluation time	Moderate-to-severe anaemia adjusted OR [a] (95% CI); p-value	Population attributable fraction (95% CI)
Iron deficiency [b]	Enrolment	3·04 (1·74, 5·33); <0·001	62% (37, 78)
	Birth	6·81 (2·23, 20·81); 0·001	84% (50, 95)
	6 months postpartum	4·06 (2·34, 7·03); <0·001	44% (22, 65)
	12 months postpartum	2·36 (1·22, 4·57); 0·01	30% (5, 57)

CI- Confidence Interval.

[a] Adjusted odds ratios for moderate-to-severe anaemia, were derived from multivariable logistic mixed-effects models with a random effect for the individual-specific intercept. Models included an interaction term included between exposure and time (enrolment, birth, 6 months postpartum and 12 months postpartum) and included age, mid-upper arm circumference, gravidity, smoking status, residence, fever and genetic polymorphisms.

Moderate-to-severe anaemia defined as haemoglobin <100g/l in pregnancy and haemoglobin <110g/l postpartum.

[b] Women classified as iron deficient: ferritin <15µg/l; replete: ferritin ≥15µg/l & CRP≤10mg/l in pregnancy and ferritin ≥15µg/l & CRP≤5mg/l postpartum.

Supplementary Table 9: Associations between lagged iron status and maternal haemoglobin levels (g/l) over the entire study period. Related to Table 2.

	Unadjusted estimated mean Hb difference (95% CI); p-value	Adjusted estimated mean Hb difference (95% CI); p-value [a]
Lagged ferritin (log$_2$(μg/l)) [b]	1·33(0·57, 2·09); 0·001	0·31 (-0·64, 1·27); 0·52
Lagged Iron deficient vs. replete [c]	-2·91 (-5·98, 0·15); 0·06	1.79 (-2·82, 6·37); 0·45

Unadjusted and adjusted estimated mean differences were derived from linear mixed-effect models with a random effect for the individual-specific intercept.

[a] Included one of the iron deficiency and lagged counterparts; along with initial haemoglobin measure, time, age, mid-upper arm circumference (MUAC), gravidity, smoking status, residence, fever, *Plasmodium* spp. infection and red blood cell polymorphisms.

[b] Ferritin transformed to log base-2 due to positively skewed distribution, thus the coefficient represents the

change associated with a two-fold increase in ferritin.

[c] Women classified as iron deficient: ferritin <15μg/l in pregnancy and postpartum. Women classified as iron

replete: ferritin ≥15μg/l and CRP≤10mg/l in pregnancy and ferritin ≥15μg/l and CRP≤5mg/l postpartum.

Supplementary Table 10: Associations between lagged iron status and maternal anaemia over the entire study period. Related to Table 2.		
	Unadjusted anaemia odds ratio (95% CI); p-value	Adjusted anaemia odds ratio (95% CI); p-value [a]
Lagged ferritin (log$_2$(μg/l)) [b]	0·90 (0·78, 1·03); 0·12	1·09 (0·94, 1·27); 0·26
Lagged Iron deficient vs. replete [c]	1·12 (0·68, 1·85); 0·65	0·62 (0·28, 1·40); 0·25

Unadjusted and adjusted odds ratios were derived from logistic mixed-effect models with a random effect for the individual-specific intercept.

[a] Included one of the iron deficiency and lagged counterparts; along with initial anaemia status, time, age, mid-upper arm circumference (MUAC), gravidity, smoking status, residence, fever, *Plasmodium* spp. infection and red blood cell polymorphisms.

Anaemia defined as haemoglobin <110g/l in pregnancy and haemoglobin <120g/l in the postpartum period.

[b] Ferritin transformed to log base-2 due to positively skewed distribution, thus the odds ratio represents the

change associated with a two-fold increase in ferritin.

[c] Women classified as iron deficient: ferritin <15μg/l in pregnancy and postpartum. Women classified as iron

replete: ferritin ≥15μg/l and CRP≤10mg/l in pregnancy and ferritin ≥15μg/l and CRP≤5mg/l postpartum.

Supplementary Table 11: *P. falciparum* and *P. vivax* qPCR reagent and cycling conditions. Related to Method details in STAR Methods.

µL/reaction		Cycling parameters	
Taqman master mix	5	50°C 2 minutes	
Forward primer	0.4	95°C 10 minutes	
Reverse primer	0.4	95°C 15 second	
Probe	0.15	58°C 60 seconds	} 45 cycles
DNase free water	1.05	40°C 60 seconds	
DNA	3		

P. falciparum: Plasmodium falciparum. P. vivax: Plasmodium vivax. qPCR: quantitative polymerase chain reaction. DNase: deoxyribonuclease. DNA: deoxyribonucleic acid.

Supplementary Table 12: Red blood cell polymorphism PCR reagent, cycling and gel conditions. Related to Method Details in STAR Methods.

Polymorphism	Reagent µL/reaction		Cycling parameters	Gel conditions
α^+- thalassemia	Taq polymerase	0.1	98°C 3 minutes	1.5% agarose gel
	α2/3.7 Forward primer	0.4	98°C 10 seconds	100V for 1.5 hours
	α2 Reverse primer	0.1	65°C 30 seconds	
	3.7 Reverse primer	0.6	60°C 50 seconds 38 cycles [a]	
	4.2 Forward primer	0.4	72°C 90 seconds	
	4.2 Reverse primer	0.4	72°C 5 minutes	
	PCR buffer	2		
	dNTPs	0.25		
	Betaine	2		
	DMSO	0.5		
	DNase free water	2.25		
	DNA	1		
CR1 deficiency (exon 22)	Taq polymerase	0.2	94°C 60 seconds	2% agarose gel
	Forward primer	0.2	94°C 15 seconds	80V for 1.5 hours
	Reverse primer	0.2	63°C 15 seconds 35 cycles	
	PCR buffer	1	72°C 90 seconds	
	dNTPs	0.2	72°C 10 minutes	
	MgCl$_2$	0.5		
	DNase free water	6.7		
	DNA	1		
CR1 deficiency (intron 27)	Taq polymerase	0.2	94°C 60 seconds	2% agarose gel
	Forward primer	0.2	94°C 15 seconds	80V for 1.5 hours
	Reverse primer	0.2	54°C 15 seconds 35 cycles	
	PCR buffer	1	72°C 90 seconds	
	dNTPs	0.2	72°C 10 minutes	
	MgCl$_2$	0.5		
	DNase free water	6.7		
	DNA	1		
SAO	Taq polymerase	0.2		

	Forward primer	0.2	95°C 2 minutes		1% agarose gel
	Reverse primer	0.2	95°C 15 seconds	⎤	80V for 1.5 hours
	PCR buffer	1	70°C 15 seconds	35 cycles	
	dNTPs	0.2	72°C 90 seconds	⎦	
	MgCl$_2$	0.5	72°C 10 minutes		
	DNase free water	6.7			
	DNA	1			

[a] Touchdown cycling used: temperature decreased by 0.5˚C/cycle for the first 8 cycles.

PCR: polymerase chain reaction. dNTP: deoxynucleoside triphosphates. DMSO: dimethyl sulfoxide. DNase: deoxyribonuclease. DNA: deoxyribonucleic acid.